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Water quality — Guidelines for quantitative sampling and sample processing of marine soft-bottom macrofauna

Qualité de l'eau — Lignes directrices pour l'échantillonnage quantitatif et le traitement d'échantillons de la macrofaune marine des fonds meubles

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2, www.iso.org/directives.

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The committee responsible for this document is ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

This second edition cancels and replaces the first edition (ISO 16665:2005), which has been technically revised.

Introduction

Analysis of macrofaunal communities in soft-bottom sediments is an integral part of marine environmental assessment. The faunal composition, in terms of both the species present and their relative abundance, reflects integrated environmental conditions in the survey area over a period of time. The composition and structure of soft-bottom macrofaunal communities therefore can be used to characterize environmental conditions and estimate the extent of environmental impact.

Characterization of environmental conditions is usually based on quantitative methods, in this case by relating the numbers of species and individuals captured to a known area of sea floor. For accurate data interpretation, it is essential to add information on the geophysicochemical characteristics or properties of the water masses and bottom sediments, including nutrients, oxygenation, and redox state where appropriate.

For effective data utilization and quality assurance (QA) of the work carried out, it is beneficial and may be essential (depending on the individual survey aims) that surveys be intercomparable temporally, spatially, and between operators. This International Standard contributes to ongoing work on QA of data from soft-bottom macrofaunal surveys. These guidelines primarily aim to assist in standardizing monitoring surveys carried out for commercial purposes or in connection with the EU Water Framework Directive. For this reason, detailed specifications are given in areas of consequence for data intercompatibility.

Where appropriate, cost–benefit issues have been taken into consideration, and accepted minimal requirements for general environmental impact assessment have been given. The cited minimum requirements for accuracy are not intended to satisfy research needs or to provide a full ecological understanding of the sampling area. Designers of programmes for research or other studies requiring a detailed knowledge of soft-bottom macrofauna should consult the guidelines given in Reference [13] for decisions on survey design and sampling frequency.

This International Standard applies to all areas of the sea floor where it is possible to collect faunal samples by a grab or coring device. For practical reasons, this applies to animals retained on a mesh screen of 0,5 mm or 1 mm aperture size.

The sensitivity of the method, here defined as detection of faunal disturbance, change in taxon composition or faunal mapping, is dependent on the survey design, the type of environmental influences present in the area and on the level of competence or standardization of the personnel.

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WARNING — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the employer or user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document be carried out by suitably trained staff.

1 Scope

This International Standard provides guidelines on the quantitative collection and processing of subtidal soft-bottom macrofaunal samples in marine waters.

This International Standard encompasses:

a) development of the sampling programme;

b) requirements for sampling equipment;

c) sampling and sample treatment in the field;

d) sorting and species identification;

e) storage of collected and processed material.

This International Standard does not specifically address the following, although some elements may be applicable:

— bioassay sub-sampling;

— deep water (>750 m) or offshore sampling;

— *in situ* faunal studies, e.g. recolonization assays;

— non-benthic organisms caught in the sampling device;

— estuarine sampling;

— intertidal sampling;

— meiofaunal sampling and analysis (see Reference [9]);

— sampling by dredge and sledge;

— self-contained underwater breathing apparatus (SCUBA) sampling;

— statistical design.

Accuracy of position fixing is determined by the geographical area, equipment used and survey objective.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1 Ecological or biological concepts

2.1.1
benthic
dwelling at the bottom of an aquatic environment

2.1.2
benthic macrofauna
bottom-dwelling animals retained on a mesh screen of 0,5 mm or 1 mm aperture size

2.1.3
receiving water body
recipient
recipient water body
water body which receives an input of material of either natural or anthropogenic origin

Note 1 to entry: The term often appears in the context of organic enrichment by, for example, effluent from municipal waste water outlets or industrial processed water. The macrofaunal part of receiving water body surveys describe the state of organic enrichment in a given area.

[SOURCE: ISO 5667-19:2004,[3] definition 3.4, modified — in the Note to entry, "contamination" has been replaced by "organic enrichment", and it is further specified that the terms apply to the macrofaunal part of receiving water body surveys]

2.1.4
soft bottom

areas of sea floor consisting of loose deposited particles including clay, mud, sand and gravel, shells and maerl, also including mixed substrata with gravels, small stones and pebbles scattered on a bed of finer material, but excluding cobbles

2.1.5

soft-bottom fauna
animals living on, or completely or partially buried in, soft-bottom sediments

2.1.6
sublittoral
portion of the shore which is either totally immersed or only uncovered by the receding tide infrequently and then for very short period (i.e. below the littoral zone)

2.2 Surveys and samples

2.2.1
baseline survey
environmental impact assessment
survey with emphasis on characterization and description of biotic and abiotic conditions in the survey area, which provides the basis for future monitoring and/or follow-up surveys

[SOURCE: ISO 5667-19:2004,[3] definition 3.2, modified — "classification" changed to "characterization"; "biotic and abiotic" added]

2.2.2
reference station
one or more sampling stations chosen to represent environmental conditions in a given area, i.e. free from direct anthropogenic influences

2.2.3
reference collection
collection of identified specimens, used for reference purposes

Note 1 to entry: Institute reference collections are usually verified by an appropriate and approved taxonomic specialist. In addition, individual identifiers may keep a personal collection and/or some surveys require contract- or area-specific reference collections.

2.2.4
replicate samples
series of samples taken in the same time frame, at the same sampling station, in the same manner for statistical validity and comparison

[SOURCE: ISO 5667-19:2004,[3] definition 3.6, modified — "simultaneously" has been changed to "in the same time frame"; "for statistical validity and comparison" added]

Note 1 to entry: Replicate samples can include sets of sub-samples taken from a larger sample.

2.2.5
sampling station
precise location where samples are collected

Note 1 to entry: A sampling station is defined by its geographical position (latitude, longitude), its depth (relative to chart datum and normalized to mean low water as given in tide tables) and any other invariant or physical conditions. The station is delineated using the given level of precision. In cases of doubt, when revisiting sampling stations, emphasis should be placed on landmarks and water depth.

2.2.6
sub-sample
ideally representative portion removed from a sample, taken for separate analysis

[SOURCE: ISO 5667-19:2004,[3] definition 3.7, modified — "ideally" and "taken for separate analysis" added]

3 Strategies and objectives for soft-bottom faunal surveys

3.1 Sampling programme and plan

The design of the sampling programme depends on the detailed aims of the survey and the required power of the data. The programme should be developed with regard to local topographical and hydrographic conditions in the survey area, information on local contamination sources, and knowledge from previous surveys, if any. The number of sampling stations, their positions and numbers of replicate samples to be taken at each sampling station should be established prior to the initiation of the survey. The design of the programme has a strong influence on the options for data treatment and statistical analyses. Prior considerations about data treatment and reporting should therefore be made. Quality assurance (QA) procedures should be incorporated at this stage.

For guidance and considerations of sampling and statistical design, see Reference [13].

3.2 Positioning of sampling stations

3.2.1 General

Sampling stations should be located to satisfy predefined requirements, bearing in mind the objectives of the study and the likely scale of natural variability in the biota.

Sampling stations should, for monitoring purposes (except for biodiversity studies — see the following), preferably be positioned in areas of homogenous sandy or muddy bottom sediments. Certain bottom types where it is difficult to obtain good quality samples, e.g. in sediments containing large amounts of stones, hard gravel, twigs and similar objects, should be avoided. However, it may be possible for a diver

to sample pockets of sediment in such areas. Alternatively, supplementary semiquantitative techniques may be used, e.g. underwater photography, video, remotely operated vehicle (ROV) or benthic dredging. Consult EN 16260[6] for guidance on visual seabed surveys.

In special cases, where habitats within the sampling area vary strongly, different sampling techniques may be combined, but generally the same gear should be used for all sampling in one survey.

For biodiversity studies, various bottom types should be included, as appropriate to the aims of the programme.

Sampling stations can be positioned according to one, or combinations of, the following strategies:

— station network, see 3.2.2;

— randomly, see 3.2.3;

— stratified, see 3.2.4;

— transect, see 3.2.5;

— single-spot sampling, see 3.2.6.

3.2.2 Station network

Sampling stations are arranged in a regular grid-like pattern. This arrangement is appropriate for overview surveys and for mapping of distribution of factors of interest, e.g. zone of influence around point source discharges. The survey area should be one of topographic homogeneity, but some adjustments can be made according to local conditions, e.g. in fjords and coastal waters with smaller variations in depth.

3.2.3 Random or scattered sampling

In special circumstances, sampling stations may be positioned randomly or scattered. An example of this is when no previous knowledge of the area is available as a guide to appropriate stratification, or when an unbiased value for a whole area is desired.

3.2.4 Stratified sampling

Sampling stations are arranged within locally homogeneous subdivisions of the survey area. The subdivisions (strata) may be delineated according to depth, sediment types or other factors that vary across the survey area. Stratification is appropriate in cases where habitat variability can confound patterns of interest. Within-strata stations may be placed in a network, e.g. for zone-of-influence mapping, or randomized for description of “average“ characteristics of the strata. Echo-sounders or appropriate ground discrimination tools should be used.

3.2.5 Transect sampling

Sampling stations are arranged along linear transects. One approach is to place stations along a known or anticipated gradient of a factor of interest in a sub-area of minimum habitat variability. Such sampling is, for example, applicable to trace effects of point-source discharges by establishing the transect in the main current direction from the source. Another rather different approach is to place stations across possible habitat gradients when it is not feasible or appropriate to work in strata.

3.2.6 Single-spot (station) sampling

This applies when a small number of stations are placed according to individual assessment. In fjordic or sill-influenced systems, where eutrophication or chemical contamination is suspected or investigated, sampling stations may be positioned in the deepest parts of the survey area (depressions, basins), where the earliest signs of disturbance are often seen.

However, no formal statistical comparison among areas is possible based on single stations. This is regarded as an undesirable design, only to be used either when it is just the station in itself that is interesting or when the limitation of available resources makes it impossible to sample several stations.

3.3 Reference stations

For surveys carried out in disturbed areas or those believed to be impacted in some way, one or more reference stations should be chosen beyond the affected area. The reference stations should, as far as possible, be representative of conditions unaffected by effluent sources and allow assessment of natural temporal and spatial variations in the soft-bottom faunal communities. Reference stations should be used in surveys where special circumstances demand direct comparison of the fauna with that beyond the disturbed or affected area, or where knowledge of the extent of natural variation is required.

Reference stations should be located in conditions as similar as possible to those at the regular sampling stations, i.e. similar depth and sediment type. Multiple reference stations are particularly important in heterogeneous areas.

Statistical considerations and the required precision of results dictate the number of reference stations and sample replicates required.

NOTE Some surveys demand a higher number of sample replicates at reference stations than at "ordinary" stations.

3.4 Types of survey

3.4.1 General

Surveys may be divided into three main categories (see Table 1) according to the objectives.

Table 1 — Overview of main categories of survey type

Survey type	Objectives	User group	Precision of results
Pilot survey	To give a general overview of bottom and faunal conditions. To provide a simple rapid assessment or to give basic information for designing more detailed sampling programmes	Regulatory authorities and consultancies. Research use as precursor to larger programme	Low
Baseline survey or environmental impact assessment	To characterize conditions in a given area. To map or identify the impact of point-source discharges (spatial extent and intensity). To compare faunal composition with specified assessment criteria or simply with other representative areas	Mainly regulatory authorities and consultancies. Research use for mapping, succession or recolonization, or gradient studies	Medium to high, depending on individual requirements
Temporal trend monitoring	To describe changes in benthic fauna over time, either for detecting change in biodiversity or as applied to environmental conditions	Mainly regulatory authorities and consultancies. Research use for environmental and biodiversity changes over time (also applied to climate monitoring)	Medium to high, depending on individual requirements

Precision of results refers to the expected accuracy of the data obtained, i.e. how representative the samples are of the environmental conditions. Precision of results is less in heterogeneous relative to homogeneous sediments or water depth across a sampling area. Therefore, to achieve the same

precision, heterogeneous sediments require higher numbers of sampling stations and/or replicate samples relative to homogeneous sediments. In addition, precision varies depending on whether the samples are processed quantitatively or semiquantitatively. The required precision and therefore the sampling and processing intensity is determined by the individual aims of the survey.

Note that the different survey types may supplement each other. For example:

— a pilot survey may provide information needed to design a sampling programme for a baseline survey or environmental impact assessment;

— any of the surveys when repeated in the same manner and at the same time of year may provide temporal trend data.

3.4.2 Pilot survey

This is an initial assessment of faunal conditions in the bottom sediments in an area where the source of the impact is not known or where there are no existing data from the area. The survey allows a coarse assessment of conditions and can provide the basis for development of a sampling programme for applied surveys, e.g. baseline or environmental impact assessment surveys as well as long-term surveillance by temporal trend monitoring. The requirements for equipment, sampling methodology, and repeatability are usually relatively simple, see Table 2.

Table 2 — Strategy and design for pilot surveys

Sampling devices	Usually grab or box corer, preferably supplemented by use of a benthic dredge. If appropriate, also other semiquantitative techniques may be used (such as underwater photography, ROV, video or acoustic ground discrimination tools).
Strategy for sampling stations	May be one or a combination of strategies outlined in 4.2
Minimum requirements for faunal assessment	Minimum requirements depend on purpose of survey. If carried out to identify best sampling stations for future programme, a minimum of semiquantitative assessment of benthic fauna should be done at all stations (at least presence and relative abundance of the major animal taxa), preferably also identification of large, abundant or otherwise prominent organisms. If pilot survey required to make firm statements about environmental disturbance, quantitative sampling is recommended.
Optional sampling	Additional samples from priority stations (as assessed by visual observations or physico-chemical data obtained during sampling or other documented or anecdotal information) may be retained for later quantitative processing.
Field documentation required	Field log of sampling conditions and sediment description (see 4.1.)
Reference station requirements	Should also be sampled, unless previous data exist to assess the status of reference areas.

Pilot surveys can have another important use, namely to help design the size and calculate statistical power for future monitoring programmes. For this purpose, the pilot study should resemble the planned monitoring programme as much as possible in terms of the spatial and temporal arrangement of samples.

A pilot survey generally requires relatively few samples. For applied purposes, the sampling area is chosen in accumulation areas rather than where net erosion takes place. Sampling stations may be positioned at random or in a grid. If the objective is to assess the faunal assemblages across an area at large, samples should be taken in both deep and shallow water. The sampling area should cover as much of the survey area as possible.

In addition to quantitative faunal sampling, dredging should be carried out to collect the rare, large and more mobile taxa not adequately sampled by remote quantitative methods. Especially in regions with varying sea floor topography and open to wind and currents, an ROV or sledge-mounted video reconnaissance is recommended to determine the extent of sediment and faunal patchiness (can occur

in areas of both coarse and fine sediments). If appropriate, acoustic ground discrimination techniques may also be used to provide additional information.

Strategy and design for pilot surveys are summarized in Table 2.

3.4.3 Baseline survey or environmental impact assessment

This is a survey widely carried out for applied research or commercial surveys, generally either where a known source of impact exists or before effluent discharge is established. Such surveys may also be carried out for biodiversity research or where an area needs to be characterized biologically. The aim is to document faunal conditions and/or map the spatial extent of biological impact. Such surveys can be carried out using relatively simple methodology, but usually there are specified requirements for the methodology and procedures to be used.

Where external reference or survey data exist, these should be used to help plan the survey programme and to assess overall impact, where appropriate. See also 4.4 for comments on supplementary non-quantitative sampling.

Strategy and design for baseline surveys or environmental impact assessment are summarized in Table 3.

Table 3 — Strategy and design for baseline surveys or environmental impact assessment

Sampling devices	Usually grab or box corer, preferably supplemented by a benthic dredge. If appropriate, also other semiquantitative techniques may be used (such as underwater photography, ROV, video or acoustic ground discrimination tools).
Strategy for sampling stations	Sampling stations positioned according to aims of survey Grid or transect sampling; stations positioned in relation to known discharge points if applicable. Stratified random sampling may also be applied according to the knowledge of expected distribution of impacts. Likely impact distribution can be determined by assessing the degree of impact in relation to local hydrography and bottom topography. If intended to detect diffuse effluent or to monitor environmental change, one station may be placed in the deepest part of the survey area (where impacted conditions often first appear). In some cases, a follow-up survey can be carried out using fewer sample replicates or sampling stations than the initial thorough environmental description. If the samples are used for legislative purpose, the required precision of results (or statistical power) should be determined, and the number of replicate samples taken adjusted as appropriate. If necessary, the number of replicate samples to be used for the analyses can be determined by calculating taxon-area curves
Minimum requirements for faunal assessment	Usually three or more, replicates are processed quantitatively, depending on statistical requirements. Faunal assessment may focus on individual taxa, groups of taxa or community-based assessment. For impact assessment, larger-scale effluents demand a more extensive station network and statistical power than small-scale effluents
Optional sampling	Contingency replicates may be collected from priority stations (assessed as for pilot survey) to be processed later if required
Field documentation required	Field log of sampling conditions and sediment description (see 4.1.)
Reference station requirements	Reference station(s) should be established in cases where environmental impacts are expected. In areas of strong impact gradients, one reference station may be sufficient. Where there is much natural variation in conditions (heterogeneous bottom) and/or only low to moderate impacts, two or more reference stations are recommended. If the end-points of transects are demonstrated outside the zone of impact, these may act as reference stations. Where standards of "pass/fail" have already been established for the area, reference stations may not be required. To assess possible overall impact in the area studied, external reference data across a wider area are recommended (can encompass new or existing data)